03/07/98

"Express Mail" mailing label number ____ 001 831 662 US I hereby certify that this document and referenced attachments are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR § 1.10, addressed to: Assistant Commissioner for Patents, Box Sequence, Washington, D.C. 20231 on 3/6/98

By: E. Durrell

Printed: E. Durrell

DOCKET #PF-0484 US

Box SEQ

Assistant Commissioner for Patents
Box Sequence
Washington, D.C. 20231

Transmitted herewith for filing is the patent application of:

Inventors: Jennifer L. Hillman; Preeti Lal; Y. Tom Tang; Henry Yue; Neil C. Corley

Title: **KINESIN LIGHT CHAIN HOMOLOG**

Enclosed are:

X Return postcard;
X 54 Pages of Specification;
X 5 Pages of Sequence Listing;
X 3 Pages of Claims;
X 1 Page of Abstract;
X 10 Pages of Figures (Figs. 1A, 1B, 1C, 1D, 1E, 1F, 1G, 2A, 2B, 2C);
X 4 Pages - **Executed** Declaration and Power of Attorney; and
X 1 Page of Sequence Listing Statement and one (1) Computer-Readable Diskette.

Fee Calculation - The fee has been calculated as follows:

CLAIMS AS FILED (fees computed under § 1.9(f))

Claims	Number Filed	Minus	Number Extra	Other Than Small Entity Rate	Fee	Basic Fee $790.00
Total Claims	21	-20	1	x $22	22	$ 22.00
Indep. Claims	2	-3	0	x $82	0	$0
__ Multiple Dependent Claim(s), if any			+ $270			$0

TOTAL FILING FEE $ 812.00

The Commissioner is hereby authorized to charge Deposit Account No. **09-0108** in the amount of **$ 812.00**.
The Commissioner is hereby authorized to charge any additional fees required under 37 C.F.R. § 1.16 and 1.17, or credit any overpayment to Deposit Account No. **09-0108. A duplicate of this sheet is enclosed.**

Respectfully submitted,

INCYTE PHARMACEUTICALS, INC.

Date: March 6, 1998

Sheela Mohan-Peterson

Sheela Mohan-Peterson
Reg. No. 41,201

3174 Porter Drive
Palo Alto, California 94304
~~Phone: (650) 855-0555~~
Fax: (650) 845-4166

DOCKET #PF-0484 US

"Express Mail" mailing label number EL 001 831 662 US I hereby certify that this document and referenced attachments are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR § 1.10, addressed to: Assistant Commissioner for Patents, Box Sequence, Washington, D.C. 20231 on 3/6/98

By: E. Durrell Printed: E. Durrell

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Hillman et al.

Title: **KINESIN LIGHT CHAIN HOMOLOG**

Serial No.: To Be Assigned Filing Date: Herewith

Examiner: To Be Assigned Group Art Unit: To Be Assigned

Assistant Commissioner for Patents
Box Sequence
Washington, D.C. 20231

SUBMISSION UNDER 37 CFR § 1.821-1.825 SEQUENCE LISTING

Sir:

In accordance with the requirements of 37 CFR § 1.821-1.825, Applicants hereby submit one diskette containing the computer-readable information for the Sequence Listing of the above-identified application. The diskette complies with the requirements of 37 CFR § 1.824 and is IBM PC compatible using a PC-DOS/MS-DOS 6.2 operating system with Fastseq software version 2.0.

Contained within the application, as filed, just before the claim section, is the Sequence Listing paper copy of the sequences disclosed in the application.

The content of the Sequence Listing paper copy is identical to the computer-readable copy, as required under 37 CFR § 1.821(f).

Respectfully submitted,

INCYTE PHARMACEUTICALS, INC.

Sheela Mohan-Peterson

Date: March 6, 1998

Sheela Mohan-Peterson
Reg. No. 41,201

3174 Porter Drive
Palo Alto, California 94304
Phone: (650) 855-0555
Fax: (650) 845-4166

KINESIN LIGHT CHAIN HOMOLOG

FIELD OF THE INVENTION

This invention relates to nucleic acid and amino acid sequences of a kinesin light chain homolog and to the use of these sequences in the diagnosis, treatment, and prevention of neurological, reproductive, and cell proliferative disorders.

BACKGROUND OF THE INVENTION

Translocation of components within cells is critical for maintaining cell structure and function. Cellular components such as proteins and membrane-bound organelles are transported along well-defined routes to specific subcellular compartments. Intracellular transport mechanisms utilize microtubules, filamentous polymers that serve as tracks that guide the movement of molecules. Transportation itself is driven by the microtubule-based motor proteins, kinesin and dynein. These proteins use the energy derived from ATP hydrolysis to power their movement unidirectionally along microtubules, and they carry molecular cargo which they release upon reaching their destinations.

Kinesin defines a large conserved family of over 50 proteins that can be classified into at least 8 subfamilies based on primary amino acid sequence, domain structure, velocity of movement, and cellular function. (Reviewed in Moore, J. D. and Endow, S. A. (1996) Bioessays 18:207-219; and Hoyt, A. M. (1994) Curr. Opin. Cell Biol. 6:63-68.) The prototypical kinesin molecule is involved in the transport of membrane-bound vesicles and organelles. This function is particularly important for axonal transport in neurons. Protein-containing vesicles are constantly transported from the neuronal cell body along microtubules that span the length of the axon leading to the synaptic terminal. Failure to supply the synaptic terminal with these vesicles blocks the transmission of neural signals. In the fruit fly Drosophila melanogaster, for example, mutations in kinesin cause severe disruption of axonal transport in larval nerves which leads to progressive paralysis. (Hurd, D. D. and Saxton, W. M. (1996) Genetics 144:1075-1085.) This phenotype mimics the pathology of some vertebrate motor neuron diseases, such as amyotrophic lateral sclerosis (ALS). In addition to axonal transport, kinesin is also important in all cell types for the transport of vesicles from

the Golgi complex to the endoplasmic reticulum. This role is critical for maintaining the identity and functionality of these secretory organelles.

Members of the more divergent subfamilies of kinesin are called kinesin-related proteins (KRPs), many of which function during mitosis in diverse eukaryotes such as yeast, frog, fruit fly, and human. Some KRPs are required for assembly of the mitotic spindle. In vivo and in vitro analyses suggest that these KRPs exert force on microtubules that comprise the mitotic spindle, resulting in the separation of spindle poles. Phosphorylation of the KRP tail domain is required for this activity. Failure to assemble the mitotic spindle results in abortive mitosis and chromosomal aneuploidy. In addition, a unique KRP, centromere protein E (CENP-E), localizes to the kinetochore of human mitotic chromosomes and may play a role in their segregation to opposite spindle poles. Other KRPs are involved in various developmental processes. For example, in the fruit fly, KRPs are involved in the movement and behavior of sperm and egg nuclei following fertilization; in the regulation of factors required for cell fate specification and pattern formation in the embryo; and, possibly, in the localization of RNA encoding developmental morphogens in the embryo. (Williams, B.C. et al. (1997) Development 124:2365-2376; and Sisson, J. C. et al. (1997) Cell 90:235-245.)

The prototypical kinesin molecule is a heterotetramer comprised of two heavy polypeptide chains (KHCs) and two light polypeptide chains (KLCs). KHC is about 1000 amino acids in length, and KLC is about 550 amino acids in length. Two KHCs dimerize in register to form a rod-shaped molecule with three distinct regions of secondary structure. At one end of the molecule is a globular motor domain that functions in ATP hydrolysis and microtubule binding. This domain is followed by an α-helical coiled-coil that is the region of dimerization. At the other end of the molecule is a fan-shaped tail that associates with molecular cargo. The tail is formed by the interaction of KHC C-termini with the two KLCs.

KLC has distinct structural domains that are conserved among species. For example, human and rat KLC are 569 and 556 amino acids in length, respectively, and share 98% amino acid identity within the first 546 amino acids. (Cabeza-Arvelaiz, Y. et al. (1993) DNA Cell Biol. 12:881-892; and Cyr, J. L. et al. (1991) Proc. Natl. Acad. Sci. USA 88:10114-10118.) Overall, KLC is predominantly hydrophilic with no major hydrophobic domains. The first 159 amino acids are predicted to form α-helices, and 15 heptad repeat sequences are

found within this region from amino acid residues 45-150. These heptad repeats contain periodically spaced hydrophobic residues that enable packing of α-helices into a coiled-coil structure. In this manner, KLC may interact not only with the C-terminus of KHC, but also with a portion of the KHC α-helical region. Within the region from amino acid residues 234-401 are four imperfect tandem repeats of 42 amino acids each. Secondary structure predictions indicate that this region lacks a hydrophobic core required to form a tightly packed globular domain. A diffusible or flexible KLC structure would account for the fan-shaped appearance of the kinesin tail. After amino acid residue 546, the sequences of human and rat KLC diverge, suggesting that variations in the KLC C-terminus may influence its activity.

The precise contribution of KLC to kinesin function is unknown. However, the localization of KLC to the kinesin tail suggests that KLC may play a role in the binding or specification of molecular cargo. Furthermore, several different isoforms of KLC can be generated by alternative splicing of a single KLC messenger RNA transcript. (Cyr et al. supra.) These isoforms, in various pairwise combinations with KHC, may generate a multitude of unique kinesin molecules capable of achieving the complex and diverse functions attributed to kinesin. In addition, KLC isoforms may differentially regulate KHC enzymatic activity or may confer tissue-specificity to kinesin function.

The discovery of a new kinesin light chain homolog and the polynucleotides encoding it satisfies a need in the art by providing new compositions which are useful in the diagnosis, treatment, and prevention of neurological, reproductive, and cell proliferative disorders.

SUMMARY OF THE INVENTION

The invention is based on the discovery of a human kinesin light chain homolog, KILCH. The invention features a substantially purified polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention further provides a substantially purified variant having at least 90% amino acid sequence identity to the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1. The invention also provides an isolated and purified polynucleotide encoding the polypeptide comprising the sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention also includes an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention further provides an isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, as well as an isolated and purified polynucleotide which is complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention also provides an isolated and purified polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2 or a fragment of SEQ ID NO:2, and an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2 or a fragment of SEQ ID NO:2. The invention also provides an isolated and purified polynucleotide having a sequence complementary to the polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2 or a fragment of SEQ ID NO:2.

The invention further provides an expression vector containing at least a fragment of the polynucleotide encoding the polypeptide comprising the sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing at least a fragment of a polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO: 1 under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having the sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1 in conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to a polypeptide

comprising the sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, as well as a purified agonist and a purified antagonist of the polypeptide.

The invention also provides a method for treating or preventing a neurological disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising substantially purified polypeptide having the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention also provides a method for treating or preventing a reproductive disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising substantially purified polypeptide having the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention also provides a method for treating or preventing a cell proliferative disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising substantially purified polypeptide having the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention also provides a method for detecting a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a fragment of SEQ ID NO: 1 in a biological sample containing nucleic acids, the method comprising the steps of: (a) hybridizing the complement of the polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1 to at least one of the nucleic acids of the biological sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1 in the biological sample. In one aspect, the nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A, 1B, 1C, 1D, 1E, 1F, and 1G show the amino acid sequence (SEQ ID NO:1) and nucleic acid sequence (SEQ ID NO:2) of KILCH. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co. Ltd., San Bruno,

CA).

Figures 2A, 2B, and 2C show the amino acid sequence alignments among KILCH (2479739; SEQ ID NO:1) and human KLC (GI 307085; SEQ ID NO:3), produced using the multisequence alignment program of LASERGENE™ software (DNASTAR Inc, Madison WI).

DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

DEFINITIONS

"KILCH," as used herein, refers to the amino acid sequences of substantially purified KILCH obtained from any species, particularly a mammalian species, including bovine,

ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term “agonist,” as used herein, refers to a molecule which, when bound to KILCH, increases or prolongs the duration of the effect of KILCH. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of KILCH.

An “allele” or an “allelic sequence,” as these terms are used herein, is an alternative form of the gene encoding KILCH. Alleles may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding KILCH, as described herein, include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide the same KILCH or a polypeptide with at least one functional characteristic of KILCH. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding KILCH, and improper or unexpected hybridization to alleles, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding KILCH. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent KILCH. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of KILCH is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and

tyrosine.

The terms "amino acid" or "amino acid sequence," as used herein, refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments", "immunogenic fragments", or "antigenic fragments" refer to fragments of KILCH which are preferably about 5 to about 15 amino acids in length and which retain some biological activity or immunological activity of KILCH. Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification," as used herein, relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art. (See, e.g., Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, pp.1-5.)

The term "antagonist," as it is used herein, refers to a molecule which, when bound to KILCH, decreases the amount or the duration of the effect of the biological or immunological activity of KILCH. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules which decrease the effect of KILCH.

As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fa, $F(ab')_2$, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind KILCH polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant," as used herein, refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may

induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense," as used herein, refers to any composition containing a nucleic acid sequence which is complementary to a specific nucleic acid sequence. The term "antisense strand" is used in reference to a nucleic acid strand that is complementary to the "sense" strand. Antisense molecules may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the designation "positive" can refer to the sense strand.

As used herein, the term "biologically active," refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic KILCH, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" or "complementarity," as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" or a "composition comprising a given amino acid sequence," as these terms are used herein, refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition

may comprise a dry formulation, an aqueous solution, or a sterile composition. Compositions comprising polynucleotide sequences encoding KILCH or fragments of KILCH may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence," as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using XL-PCR™ (Perkin Elmer, Norwalk, CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte Clone using a computer program for fragment assembly, such as the GELVIEW™ Fragment Assembly system (GCG, Madison, WI). Some sequences have been both extended and assembled to produce the consensus sequence.

As used herein, the term "correlates with expression of a polynucleotide" indicates that the detection of the presence of nucleic acids, the same or related to a nucleic acid sequence encoding KILCH, by northern analysis is indicative of the presence of nucleic acids encoding KILCH in a sample, and thereby correlates with expression of the transcript from the polynucleotide encoding KILCH.

A "deletion," as the term is used herein, refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative," as used herein, refers to the chemical modification of KILCH, of a polynucleotide sequence encoding KILCH, or of a polynucleotide sequence complementary to a polynucleotide sequence encoding KILCH. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

The term "homology," as used herein, refers to a degree of complementarity. There

may be partial homology or complete homology. The word "identity" may substitute for the word "homology." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially homologous sequence or hybridization probe will compete for and inhibit the binding of a completely homologous sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% homology or identity). In the absence of non-specific binding, the substantially homologous sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" or "% identity" refer to the percentage of sequence similarity found in a comparison of two or more amino acid or nucleic acid sequences. Percent identity can be determined electronically, e.g., by using the MegAlign™ program (DNASTAR, Inc., Madison WI). The MegAlign™ program can create alignments between two or more sequences according to different methods, e.g., the clustal method. (See, e.g., Higgins, D.G. and P.M. Sharp (1988) Gene 73:237-244.) The clustal algorithm groups sequences into clusters by examining the distances between all pairs. The clusters are aligned pairwise and then in groups. The percentage similarity between two amino acid sequences, e.g., sequence A and sequence B, is calculated by dividing the length of sequence A, minus the number of gap residues in sequence A, minus the number of gap residues in sequence B, into the sum of the residue matches between sequence A and sequence B, times one hundred. Gaps of low or of no homology between the two amino acid sequences are not included in determining percentage similarity. Percent identity between nucleic acid sequences can also be counted or calculated by other methods known in the art, e.g., the Jotun Hein method. (See, e.g., Hein, J. (1990) Methods Enzymol. 183:626-645.) Identity between sequences can

also be determined by other methods known in the art, e.g., by varying hybridization conditions.

"Human artificial chromosomes" (HACs), as described herein, are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance. (See, e.g., Harrington, J.J. et al. (1997) Nat Genet. 15:345-355.)

The term "humanized antibody," as used herein, refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization," as the term is used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

As used herein, the term "hybridization complex" as used herein, refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C_0t or R_0t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" or "addition," as used herein, refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, to the sequence found in the naturally occurring molecule.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

The term "microarray," as used herein, refers to an arrangement of distinct polynucleotides arrayed on a substrate, e.g., paper, nylon or any other type of membrane, filter, chip, glass slide, or any other suitable solid support.

The terms "element" or "array element" as used herein in a microarray context, refer

to hybridizable polynucleotides arranged on the surface of a substrate.

The term "modulate," as it appears herein, refers to a change in the activity of KILCH. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of KILCH.

The phrases "nucleic acid" or "nucleic acid sequence," as used herein, refer to an oligonucleotide, nucleotide, polynucleotide, or any fragment thereof, to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. In this context, "fragments" refers to those nucleic acid sequences which are greater than about 60 nucleotides in length, and most preferably are at least about 100 nucleotides, at least about 1000 nucleotides, or at least about 10,000 nucleotides in length.

The terms "operably associated" or "operably linked," as used herein, refer to functionally related nucleic acid sequences. A promoter is operably associated or operably linked with a coding sequence if the promoter controls the transcription of the encoded polypeptide. While operably associated or operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements, e.g., repressor genes, are not contiguously linked to the encoded polypeptide but still bind to operator sequences that control expression of the polypeptide.

The term "oligonucleotide," as used herein, refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or microarray. As used herein, the term "oligonucleotide" is substantially equivalent to the terms "amplimer," "primer," "oligomer," and "probe," as these terms are commonly defined in the art.

"Peptide nucleic acid" (PNA), as used herein, refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA and RNA and stop transcript elongation, and may be pegylated to extend their

lifespan in the cell. (See, e.g., Nielsen, P.E. et al. (1993) Anticancer Drug Des. 8:53-63.)

The term "sample," as used herein, is used in its broadest sense. A biological sample suspected of containing nucleic acids encoding KILCH, or fragments thereof, or KILCH itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a solid support; a tissue; a tissue print; etc.

As used herein, the terms "specific binding" or "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

As used herein, the term "stringent conditions" refers to conditions which permit hybridization between polynucleotide sequences and the claimed polynucleotide sequences. Suitably stringent conditions can be defined by, for example, the concentrations of salt or formamide in the prehybridization and hybridization solutions, or by the hybridization temperature, and are well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

For example, hybridization under high stringency conditions could occur in about 50% formamide at about 37°C to 42°C. Hybridization could occur under reduced stringency conditions in about 35% to 25% formamide at about 30°C to 35°C. In particular, hybridization could occur under high stringency conditions at 42°C in 50% formamide, 5X SSPE, 0.3% SDS, and 200 μg/ml sheared and denatured salmon sperm DNA. Hybridization could occur under reduced stringency conditions as described above, but in 35% formamide at a reduced temperature of 35°C. The temperature range corresponding to a particular level of stringency can be further narrowed by calculating the purine to pyrimidine ratio of the nucleic acid of interest and adjusting the temperature accordingly. Variations on the above ranges and conditions are well known in the art.

The term "substantially purified," as used herein, refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

A "substitution," as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Transformation," as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "variant" of KILCH, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, LASERGENE™ software.

THE INVENTION

The invention is based on the discovery of a new human kinesin light chain homolog (KILCH), the polynucleotides encoding KILCH, and the use of these compositions for the diagnosis, treatment, or prevention of neurological, reproductive, and cell proliferative disorders.

Nucleic acids encoding the KILCH of the present invention were first identified in Incyte Clone 2479739 from the aortic smooth muscle cell line cDNA library (SMCANOT01) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:2, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 2479739 (SMCANOT01), 3044995 (HEAANOT01), 2513404 and 2514442 (LIVRTUT04), 1691055 (PROSTUT10), 1630522 (COLNNOT19), and shotgun sequences SAEA01144, SASA02596, and SAPA00507.

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1, as shown in Figures 1A, 1B, 1C, 1D, 1E, 1F, and 1G. KILCH is 619 amino acids in length and has two potential cAMP- and cGMP-dependent protein kinase phosphorylation sites at S_{519} and S_{566}; nine potential casein kinase II phosphorylation sites at S_{18}, S_{96}, T_{162}, S_{174}, S_{281}, S_{416}, T_{485}, T_{518}, and S_{611}; seven potential protein kinase C phosphorylation sites at S_{25}, S_{100}, S_{245}, S_{281}, T_{466}, S_{493}, and S_{526}; and three kinesin light chain repeat signatures from D_{278} to Q_{319}, R_{320} to Q_{361}, and R_{362} to K_{403}. As shown in Figure 2, KILCH has chemical and structural homology with human KLC (GI 307085; SEQ ID NO:3). In particular, KILCH and human KLC share 66% identity. In addition, the region of KILCH from N_{77} to L_{153} shares 83% identity with the region of human KLC that contains 11 of the 15 heptad repeats. The region of KILCH from Q_{234} to K_{403} shares 87% identity with the region of human KLC that contains four imperfect tandem repeats. Furthermore, the potential phosphorylation sites at S_{18}, S_{100}, S_{416}, T_{466}, T_{485}, and S_{493} of KILCH are conserved in human KLC. A region of unique sequence in KILCH from about amino acid 6 to about amino acid 17 is encoded by a fragment of SEQ ID NO:2 corresponding to about nucleotide 184 to about nucleotide 219. Northern analysis shows the expression of this sequence in various libraries, at least 47% are associated with cancer and cell proliferation. In particular, 24% of the libraries expressing KILCH are derived from reproductive tissue, and 17% are derived from neural tissue.

The invention also encompasses KILCH variants. A preferred KILCH variant is one which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the KILCH amino acid sequence, and which contains at least one functional or structural characteristic of KILCH.

The invention also encompasses polynucleotides which encode KILCH. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising the sequence of SEQ ID NO:2, which encodes an KILCH.

The invention also encompasses a variant of a polynucleotide sequence encoding KILCH. In particular, such a variant polynucleotide sequence will have at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding KILCH. A particular aspect of the invention encompasses a variant of SEQ ID NO:2 which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to SEQ ID NO:2. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of KILCH.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding KILCH, some bearing minimal homology to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring KILCH, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode KILCH and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring KILCH under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding KILCH or its derivatives possessing a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding KILCH and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode KILCH and KILCH derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding KILCH or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:2, or a fragment of SEQ ID NO:2, under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; Kimmel, A.R. (1987) Methods Enzymol. 152:507-511.)

Methods for DNA sequencing are well known and generally available in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp., Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE Amplification System (GIBCO/BRL, Gaithersburg, MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI Catalyst and 373 and 377 DNA Sequencers (Perkin Elmer).

The nucleic acid sequences encoding KILCH may be extended utilizing a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal primers to retrieve unknown sequence adjacent to a known locus. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) In particular, genomic DNA is first amplified in the presence of a primer which is complementary to a linker sequence within the vector and a primer specific to a region of the nucleotide sequenc. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using

reverse transcriptase.

Inverse PCR may also be used to amplify or extend sequences using divergent primers based on a known region. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) The primers may be designed using commercially available software such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN) or another appropriate program to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to 72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which may be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060.) Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable in that they will include more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and a

charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., Genotyper™ and Sequence Navigator™, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode KILCH may be used in recombinant DNA molecules to direct expression of KILCH, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced, and these sequences may be used to clone and express KILCH.

As will be understood by those of skill in the art, it may be advantageous to produce KILCH-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter KILCH-encoding sequences for a variety of reasons including, but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding KILCH may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of KILCH activity, it may be useful to encode a chimeric KILCH protein that can be recognized by a commercially

available antibody. A fusion protein may also be engineered to contain a cleavage site located between the KILCH encoding sequence and the heterologous protein sequence, so that KILCH may be cleaved and purified away from the heterologous moiety.

In another embodiment, sequences encoding KILCH may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, and Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232.) Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of KILCH, or a fragment thereof. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A Peptide Synthesizer (Perkin Elmer). Additionally, the amino acid sequence of KILCH, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g, Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1983) <u>Proteins, Structures and Molecular Properties</u>, WH Freeman and Co., New York, NY.)

In order to express a biologically active KILCH, the nucleotide sequences encoding KILCH or derivatives thereof may be inserted into appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding KILCH and appropriate transcriptional and translational control elements. These methods include <u>in vitro</u> recombinant DNA techniques, synthetic techniques, and <u>in vivo</u> genetic recombination. (See, e.g., Sambrook, J. et al. (1989) <u>Molecular Cloning, A Laboratory Manual</u>, Cold Spring Harbor Press, Plainview, NY, ch. 4, 8, and 16-17; and Ausubel, F.M. et al. (1995, and periodic supplements) <u>Current Protocols in Molecular Biology</u>, John Wiley & Sons, New York, NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding KILCH. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus (CaMV) or tobacco mosaic virus (TMV)) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

The "control elements" or "regulatory sequences" are those non-translated regions, e.g., enhancers, promoters, and 5' and 3' untranslated regions, of the vector and polynucleotide sequences encoding KILCH which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters, e.g., hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, La Jolla, CA) or pSport1™ plasmid (GIBCO/BRL), may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding KILCH, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for KILCH. For example, when large quantities of KILCH are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, multifunctional E. coli cloning and expression vectors such as Bluescript® (Stratagene), in which the sequence encoding KILCH may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of ß-galactosidase so that a hybrid protein

is produced, and pIN vectors. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) pGEX vectors (Amersham Pharmacia Biotech, Uppsala, Sweden) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH, may be used. (See, e.g., Ausubel, supra; and Grant et al. (1987) Methods Enzymol. 153:516-544.)

In cases where plant expression vectors are used, the expression of sequences encoding KILCH may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV. (Takamatsu, N. (1987) EMBO J. 6:307-311.) Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques are described in a number of generally available reviews. (See, e.g., Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.)

An insect system may also be used to express KILCH. For example, in one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The sequences encoding KILCH may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of sequences encoding KILCH will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which KILCH may be expressed.

(See, e.g., Engelhard, E.K. et al. (1994) Proc. Nat. Acad. Sci. 91:3224-3227.)

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding KILCH may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing KILCH in infected host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding KILCH. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding KILCH and its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding,

and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC, Bethesda, MD) and may be chosen to ensure the correct modification and processing of the foreign protein.

For long term, high yield production of recombinant proteins, stable expression is preferred. For example, cell lines capable of stably expressing KILCH can be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase genes and adenine phosphoribosyltransferase genes, which can be employed in *tk*⁻ or *apr*⁻ cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; and Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *npt* confers resistance to the aminoglycosides neomycin and G-418; and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-3570; Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14; and Murry, supra.) Additional selectable genes have been described, e.g., *trpB*, which allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-8051.) Visible markers, e.g., anthocyanins, ß glucuronidase and its substrate GUS, luciferase and its substrate luciferin may be used. Green fluorescent proteins (GFP) (Clontech, Palo Alto, CA) can also be used. These markers can be used not only to

identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding KILCH is inserted within a marker gene sequence, transformed cells containing sequences encoding KILCH can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding KILCH under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain the nucleic acid sequence encoding KILCH and express KILCH may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

The presence of polynucleotide sequences encoding KILCH can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding KILCH. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences encoding KILCH to detect transformants containing DNA or RNA encoding KILCH.

A variety of protocols for detecting and measuring the expression of KILCH, using either polyclonal or monoclonal antibodies specific for the protein, are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on KILCH is preferred, but a competitive binding assay may be employed. These and other assays are well described in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul, MN, Section IV; and Maddox, D.E. et al. (1983) J. Exp. Med. 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding KILCH include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding KILCH, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Pharmacia & Upjohn (Kalamazoo, MI), Promega (Madison, WI), and U.S. Biochemical Corp. (Cleveland, OH). Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding KILCH may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode KILCH may be designed to contain signal sequences which direct secretion of KILCH through a prokaryotic or eukaryotic cell membrane. Other constructions may be used to join sequences encoding KILCH to nucleotide sequences encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences, such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA), between the purification domain and the KILCH encoding sequence may be used to facilitate purification. One such expression vector provides for expression of a fusion protein

containing KILCH and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on immobilized metal ion affinity chromatography (IMAC). (See, e.g., Porath, J. et al. (1992) Prot. Exp. Purif. 3: 263-281.) The enterokinase cleavage site provides a means for purifying KILCH from the fusion protein. (See, e.g., Kroll, D.J. et al. (1993) DNA Cell Biol. 12:441-453.)

Fragments of KILCH may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, T.E. (1984) Protein: Structures and Molecular Properties, pp. 55-60, W.H. Freeman and Co., New York, NY.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of KILCH may be synthesized separately and then combined to produce the full length molecule.

THERAPEUTICS

Chemical and structural homology exists between KILCH and KLC from human (GI 307085). In addition, KILCH is expressed in neurological, reproductive, and proliferating tissues. Therefore, KILCH appears to play a role in neurological, reproductive, and cell proliferative disorders.

Therefore, in one embodiment, KILCH or a fragment or derivative thereof may be administered to a subject to treat or prevent a neurological disorder. Such disorders can include, but are not limited to, akathesia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder, catatonia, cerebral neoplasms, dementia, depression, diabetic neuropathy, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, peripheral neuropathy, multiple sclerosis, neurofibromatosis, Parkinson's disease, paranoid psychoses, postherpetic neuralgia, schizophrenia, and Tourette's disorder.

In another embodiment, a vector capable of expressing KILCH or a fragment or derivative thereof may be administered to a subject to treat or prevent a neurological disorder including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified KILCH in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a neurological disorder including, but not limited to, those

provided above.

In still another embodiment, an agonist which modulates the activity of KILCH may be administered to a subject to treat or prevent a neurological disorder including, but not limited to, those listed above.

In another embodiment, KILCH or a fragment or derivative thereof may be administered to a subject to treat or prevent a reproductive disorder. Such disorders can include, but are not limited to, abnormal prolactin production, infertility, tubal disease, ovulatory defects, endometriosis, perturbations of the estrous and menstrual cycles, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, autoimmune disorders, ectopic pregnancy, teratogenesis, breast cancer, fibrocystic breast disease, galactorrhea, abnormal spermatogenesis, abnormal sperm physiology, testicular cancer, prostate cancer, benign prostatic hyperplasia, prostatitis, and gynecomastia.

In another embodiment, a vector capable of expressing KILCH or a fragment or derivative thereof may be administered to a subject to treat or prevent a reproductive disorder including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified KILCH in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a reproductive disorder including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of KILCH may be administered to a subject to treat or prevent a reproductive disorder including, but not limited to, those listed above.

In another embodiment, KILCH or a fragment or derivative thereof may be administered to a subject to treat or prevent a cell proliferative disorder. Such disorders can include, but are not limited to, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung,

muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

In another embodiment, a vector capable of expressing KILCH or a fragment or derivative thereof may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those described above.

In a further embodiment, a pharmaceutical composition comprising a substantially purified KILCH in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of KILCH may be administered to a subject to treat or prevent a cell proliferative disorder including, but not limited to, those listed above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of KILCH may be produced using methods which are generally known in the art. In particular, purified KILCH may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind KILCH. Antibodies to KILCH may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with KILCH or with any fragment or

oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to KILCH have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of KILCH amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to KILCH may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce KILCH-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton D.R. (1991) Proc. Natl. Acad. Sci. 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. 86: 3833-3837; and Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for KILCH may also be generated. For example, such fragments include, but are not limited to, F(ab')2 fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between KILCH and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering KILCH epitopes is preferred, but a competitive binding assay may also be employed. (Maddox, supra.)

In another embodiment of the invention, the polynucleotides encoding KILCH, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding KILCH may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding KILCH. Thus, complementary molecules or fragments may be used to modulate KILCH activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding KILCH.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences

to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors which will express nucleic acid sequences complementary to the polynucleotides of the gene encoding KILCH. (See, e.g., Sambrook, supra; and Ausubel, supra.)

Genes encoding KILCH can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding KILCH. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding KILCH. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding KILCH.

Specific ribozyme cleavage sites within any potential RNA target are initially

identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding KILCH. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nature Biotechnology 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need

of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of KILCH, antibodies to KILCH, and mimetics, agonists, antagonists, or inhibitors of KILCH. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose,

sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acid. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of KILCH, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example KILCH or fragments thereof, antibodies of KILCH, and agonists, antagonists or inhibitors of KILCH, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED_{50} (the dose therapeutically effective in 50% of the population) or LD_{50} (the dose lethal to 50% of the population) statistics. The dose ratio of therapeutic to toxic effects is the therapeutic index, and it can be expressed as the ED_{50}/LD50

ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1 μg to 100,000 μg, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

DIAGNOSTICS

In another embodiment, antibodies which specifically bind KILCH may be used for the diagnosis of disorders characterized by expression of KILCH, or in assays to monitor patients being treated with KILCH or agonists, antagonists, or inhibitors of KILCH. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for KILCH include methods which utilize the antibody and a label to detect KILCH in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring KILCH, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of KILCH expression. Normal or standard values for KILCH expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to KILCH under conditions suitable for complex formation The amount of standard complex formation may be quantitated by various methods, preferably by photometric means. Quantities of KILCH expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding KILCH may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of KILCH may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of KILCH, and to monitor regulation of KILCH levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding KILCH or closely related molecules may be used to identify nucleic acid sequences which encode KILCH. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding KILCH, alleles, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably have at least 50% sequence identity to any of the KILCH encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:2 or from genomic sequences including promoters, enhancers, and introns of the KILCH gene.

Means for producing specific hybridization probes for DNAs encoding KILCH

include the cloning of polynucleotide sequences encoding KILCH or KILCH derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as ^{32}P or ^{35}S, or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding KILCH may be used for the diagnosis of a disorder associated with expression of KILCH. Examples of such a disorder include, but are not limited to, a neurological disorder such as akathesia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder, catatonia, cerebral neoplasms, dementia, depression, diabetic neuropathy, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, peripheral neuropathy, multiple sclerosis, neurofibromatosis, Parkinson's disease, paranoid psychoses, postherpetic neuralgia, schizophrenia, and Tourette's disorder; a reproductive disorder such as abnormal prolactin production, infertility, tubal disease, ovulatory defects, endometriosis, perturbations of the estrous and menstrual cycles, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, autoimmune disorders, ectopic pregnancy, teratogenesis, breast cancer, fibrocystic breast disease, galactorrhea, abnormal spermatogenesis, abnormal sperm physiology, testicular cancer, prostate cancer, benign prostatic hyperplasia, prostatitis, and gynecomastia; and a cell proliferative disorder such as arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus. The polynucleotide sequences encoding KILCH may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and ELISA assays; and in microarrays

utilizing fluids or tissues from patients to detect altered KILCH expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding KILCH may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding KILCH may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding KILCH in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of KILCH, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding KILCH, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the

disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding KILCH may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding KILCH, or a fragment of a polynucleotide complementary to the polynucleotide encoding KILCH, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of KILCH include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; and Duplaa, C. et al. (1993) Anal. Biochem. 229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No.

5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding KILCH may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, R.A. (ed.) Molecular Biology and Biotechnology, VCH Publishers New York, NY, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene encoding KILCH on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to a particular genomic region, e.g., AT to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, KILCH, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between KILCH and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with KILCH, or fragments thereof, and washed. Bound KILCH is then detected by methods well known in the art. Purified KILCH can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding KILCH specifically compete with a test compound for binding KILCH. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with KILCH.

In additional embodiments, the nucleotide sequences which encode KILCH may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

EXAMPLES

I. SMCANOT01 cDNA Library Construction

The SMCANOT01 cDNA library was constructed from an aortic smooth muscle cell line derived from explanted heart tissue obtained from a male undergoing a heart transplant.

Prior to the actual transplantation, a sample of aortic tissue was removed from the patient's heart. Smooth muscle cells were isolated from this sample and passaged in culture approximately four times.

The frozen tissue was homogenized and lysed in guanidinium isothiocyanate solution using a Brinkmann Homogenizer Polytron PT-3000 (Brinkmann Instruments, Westbury, NY). The lysate was centrifuged over a CsCl cushion to isolate RNA. The RNA was extracted with acid phenol, precipitated with sodium acetate and ethanol, resuspended in RNase-free water, and treated with DNase. The RNA was re-extracted twice with acid phenol and reprecipitated with sodium acetate and ethanol. Poly(A+) RNA was isolated using the Qiagen Oligotex kit (QIAGEN Inc, Chatsworth, CA).

Poly (A+) RNA was used to construct the SMCANOT01 cDNA library according to the recommended protocols in the SuperScript plasmid system (Catalog #18248-013, Gibco/BRL). The cDNAs were fractionated on a Sepharose CL4B column (Catalog #275105-01, Pharmacia, Piscataway, NJ), and those cDNAs exceeding 400 bp were ligated into the plasmid pINCY 1 (Incyte). pINCY 1 was subsequently transformed into DH5α™ competent cells (Catalog #18258-012, Gibco/BRL).

II. Isolation and Sequencing of cDNA Clones

Plasmid DNA was released from the cells and purified using the REAL Prep 96 plasmid kit (Catalog #26173, QIAGEN). The recommended protocol was employed except for the following changes: 1) the bacteria were cultured in 1 ml of sterile Terrific Broth (Catalog #22711, Gibco/BRL) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) after the cultures were incubated for 19 hours, the cells were lysed with 0.3 ml of lysis buffer; and 3) following isopropanol precipitation, the plasmid DNA pellets were each resuspended in 0.1 ml of distilled water. The DNA samples were stored at 4° C.

The cDNAs were sequenced by the method of Sanger et al. (1975, J. Mol. Biol. 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno, NV) in combination with Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown, MA) and Applied Biosystems 377 DNA Sequencing Systems.

III. Homology Searching of cDNA Clones and Their Deduced Proteins

The nucleotide sequences and/or amino acid sequences of the Sequence Listing were used to query sequences in the GenBank, SwissProt, BLOCKS, and Pima II databases. These databases, which contain previously identified and annotated sequences, were searched for regions of homology using BLAST (Basic Local Alignment Search Tool). (See, e.g., Altschul, S.F. (1993) J. Mol. Evol 36:290-300; and Altschul et al. (1990) J. Mol. Biol. 215:403-410.)

BLAST produced alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST was especially useful in determining exact matches or in identifying homologs which may be of prokaryotic (bacterial) or eukaryotic (animal, fungal, or plant) origin. Other algorithms could have been used when dealing with primary sequence patterns and secondary structure gap penalties. (See, e.g., Smith, T. et al. (1992) Protein Engineering 5:35-51.) The sequences disclosed in this application have lengths of at least 49 nucleotides and have no more than 12% uncalled bases (where N is recorded rather than A, C, G, or T).

The BLAST approach searched for matches between a query sequence and a database sequence. BLAST evaluated the statistical significance of any matches found, and reported only those matches that satisfy the user-selected threshold of significance. In this application, threshold was set at 10^{-25} for nucleotides and 10^{-8} for peptides.

Incyte nucleotide sequences were searched against the GenBank databases for primate (pri), rodent (rod), and other mammalian sequences (mam), and deduced amino acid sequences from the same clones were then searched against GenBank functional protein databases, mammalian (mamp), vertebrate (vrtp), and eukaryote (eukp), for homology.

Additionally, sequences identified from cDNA libraries may be analyzed to identify those gene sequences encoding conserved protein motifs using an appropriate analysis program, e.g., the Block 2 Bioanalysis Program (Incyte, Palo Alto, CA). This motif analysis program, based on sequence information contained in the Swiss-Prot Database and PROSITE, is a method of determining the function of uncharacterized proteins translated from genomic or cDNA sequences. (See, e.g., Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221; and Attwood, T. K. et al. (1997) J. Chem. Inf. Comput. Sci. 37:417-424.) PROSITE may be used

to identify common functional or structural domains in divergent proteins. The method is based on weight matrices. Motifs identified by this method are then calibrated against the SWISS-PROT database in order to obtain a measure of the chance distribution of the matches.

In another alternative, Hidden Markov models (HMMs) may be used to find protein domains, each defined by a dataset of proteins known to have a common biological function. (See, e.g., Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-2448; and Smith, T.F. and M.S. Waterman (1981) J. Mol. Biol. 147:195-197.) HMMs were initially developed to examine speech recognition patterns, but are now being used in a biological context to analyze protein and nucleic acid sequences as well as to model protein structure. (See, e.g., Krogh, A. et al. (1994) J. Mol. Biol. 235:1501-1531; and Collin, M. et al. (1993) Protein Sci. 2:305-314.) HMMs have a formal probabilistic basis and use position-specific scores for amino acids or nucleotides. The algorithm continues to incorporate information from newly identified sequences to increase its motif analysis capabilities.

IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; and Ausubel, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST are used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ™ database (Incyte Pharmaceuticals). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

The basis of the search is the product score, which is defined as:

$$\frac{\%\ \text{sequence identity} \times \%\ \text{maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be

exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of northern analysis are reported as a list of libraries in which the transcript encoding KILCH occurs. Abundance and percent abundance are also reported. Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

V. Extension of KILCH Encoding Polynucleotides

The nucleic acid sequence of Incyte Clone 2479739 was used to design oligonucleotide primers for extending a partial nucleotide sequence to full length. One primer was synthesized to initiate extension of an antisense polynucleotide, and the other was synthesized to initiate extension of a sense polynucleotide. Primers were used to facilitate the extension of the known sequence "outward" generating amplicons containing new unknown nucleotide sequence for the region of interest. The initial primers were designed from the cDNA using OLIGO 4.06 (National Biosciences, Plymouth, MN), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries (GIBCO/BRL) were used to extend the sequence. If more than one extension is necessary or desired, additional sets of primers are designed to further extend the known region.

High fidelity amplification was obtained by following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix. PCR was performed using the Peltier Thermal Cycler (PTC200; M.J. Research, Watertown, MA), beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, with the following parameters:

Step 1	94° C for 1 min (initial denaturation)
Step 2	65° C for 1 min

Step 3	68° C for 6 min
Step 4	94° C for 15 sec
Step 5	65° C for 1 min
Step 6	68° C for 7 min
Step 7	Repeat steps 4 through 6 for an additional 15 cycles
Step 8	94° C for 15 sec
Step 9	65° C for 1 min
Step 10	68° C for 7:15 min
Step 11	Repeat steps 8 through 10 for an additional 12 cycles
Step 12	72° C for 8 min
Step 13	4° C (and holding)

A 5 μl to 10 μl aliquot of the reaction mixture was analyzed by electrophoresis on a low concentration (about 0.6% to 0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products were excised from the gel, purified using QIAQuick™ (QIAGEN Inc.), and trimmed of overhangs using Klenow enzyme to facilitate religation and cloning.

After ethanol precipitation, the products were redissolved in 13 μl of ligation buffer, 1μl T4-DNA ligase (15 units) and 1μl T4 polynucleotide kinase were added, and the mixture was incubated at room temperature for 2 to 3 hours, or overnight at 16° C. Competent E. coli cells (in 40 μl of appropriate media) were transformed with 3 μl of ligation mixture and cultured in 80 μl of SOC medium. (See, e.g., Sambrook, supra, Appendix A, p. 2.) After incubation for one hour at 37° C, the E. coli mixture was plated on Luria Bertani (LB) agar (See, e.g., Sambrook, supra, Appendix A, p. 1) containing carbenicillin (2x carb). The following day, several colonies were randomly picked from each plate and cultured in 150 μl of liquid LB/2x Carb medium placed in an individual well of an appropriate commercially-available sterile 96-well microtiter plate. The following day, 5 μl of each overnight culture was transferred into a non-sterile 96-well plate and, after dilution 1:10 with water, 5 μl from each sample was transferred into a PCR array.

For PCR amplification, 18 μl of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction were added to each well. Amplification was performed using the following conditions:

Step 1	94° C for 60 sec
Step 2	94° C for 20 sec

Step 3	55° C for 30 sec
Step 4	72° C for 90 sec
Step 5	Repeat steps 2 through 4 for an additional 29 cycles
Step 6	72° C for 180 sec
Step 7	4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular weight markers. The sizes of the PCR products were compared to the original partial cDNAs, and appropriate clones were selected, ligated into plasmid, and sequenced.

In like manner, the nucleotide sequence of SEQ ID NO:2 is used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for 5' extension, and an appropriate genomic library.

VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:2 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 μCi of [γ-^{32}P] adenosine triphosphate (Amersham, Chicago, IL), and T4 polynucleotide kinase (DuPont NEN®, Boston, MA). The labeled oligonucleotides are substantially purified using a Sephadex G-25 superfine resin column (Pharmacia & Upjohn, Kalamazoo, MI). An aliquot containing 10^7 counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba1, or Pvu II (DuPont NEN, Boston, MA).

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR™ film (Kodak, Rochester, NY) is exposed to the blots to film for several hours, hybridization patterns are compared visually.

VII. Microarrays

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, supra.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE™. Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; and Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described above.

VIII. Complementary Polynucleotides

Sequences complementary to the KILCH-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring KILCH. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using Oligo 4.06 software and the coding sequence of KILCH. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit

translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the KILCH-encoding transcript.

IX. Expression of KILCH

Expression of KILCH is accomplished by subcloning the cDNA into an appropriate vector and transforming the vector into host cells. This vector contains an appropriate promoter, e.g., ß-galactosidase, upstream of the cloning site, operably associated with the cDNA of interest. (See, e.g., Sambrook, supra, pp. 404-433; and Rosenberg, M. et al. (1983) Methods Enzymol. 101:123-138.)

Induction of an isolated, transformed bacterial strain with isopropyl beta-D-thiogalactopyranoside (IPTG) using standard methods produces a fusion protein which consists of the first 8 residues of ß-galactosidase, about 5 to 15 residues of linker, and the full length protein. The signal residues direct the secretion of KILCH into bacterial growth media which can be used directly in the following assay for activity.

X. Demonstration of KILCH Activity

A blot-overlay assay for KILCH activity measures its affinity for the C-terminal tail domain of kinesin heavy chain (CTD-KHC). (Gauger, A. K. and Goldstein, L. S. B. (1993) J. Biol. Chem. 268:13657-13666.) CTD-KHC is highly conserved among various species; therefore, the source of CTD-KHC for this assay may be human, rat, sea urchin, squid, or fruit fly. CTD-KHC, which consists of approximately 300 amino acids from the KHC C-terminus, is tagged with glutathione *S*-transferase (GST). The construction, expression, and purification of this tagged protein, called CTD-KHC-GST, are achieved using recombinant DNA methods and prokaryotic systems well known to those skilled in the art. A buffered salt solution containing 0.5 μg/ml CTD-KHC-GST is applied to a KILCH blot constructed as follows: 1 μg of KILCH, either produced by recombinant methods or purified biochemically, is subjected to SDS-PAGE and transferred to nitrocellulose membrane. The blot is incubated in CTD-KHC-GST solution and washed. CTD-KHC-GST bound to KILCH is detected and quantified using anti-GST antibodies, enzyme-conjugated secondary antibodies, and chemiluminescent detection systems. The amount of CTD-KHC-GST bound to KILCH is

directly proportional to the affinity of CTD-KHC-GST for KILCH.

XI. Production of KILCH Specific Antibodies

KILCH substantially purified using PAGE electrophoresis (see, e.g., Harrington, M.G. (1990) Methods Enzymol. 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the KILCH amino acid sequence is analyzed using LASERGENE™ software (DNASTAR Inc.) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel supra, ch. 11.)

Typically, oligopeptides 15 residues in length are synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and coupled to KLH (Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

XII. Purification of Naturally Occurring KILCH Using Specific Antibodies

Naturally occurring or recombinant KILCH is substantially purified by immunoaffinity chromatography using antibodies specific for KILCH. An immunoaffinity column is constructed by covalently coupling anti-KILCH antibody to an activated chromatographic resin, such as CNBr-activated Sepharose (Pharmacia & Upjohn). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing KILCH are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of KILCH (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under

conditions that disrupt antibody/KILCH binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and KILCH is collected.

XIII. Identification of Molecules Which Interact with KILCH

KILCH, or biologically active fragments thereof, are labeled with ^{125}I Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled KILCH, washed, and any wells with labeled KILCH complex are assayed. Data obtained using different concentrations of KILCH are used to calculate values for the number, affinity, and association of KILCH with the candidate molecules.

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

#5/a

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Hillman, Jennifer L.
Lal, Preeti
Tang, Y. Tom
Yue, Henry
Corley, Neil C.

(ii) TITLE OF THE INVENTION: KINESIN LIGHT CHAIN HOMOLOG

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Incyte Pharmaceuticals, Inc.
(B) STREET: 3174 Porter Dr.
(C) CITY: Palo Alto
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94304

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: To Be Assigned
(B) FILING DATE: Filed Herewith

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Billings, Lucy J.
(B) REGISTRATION NUMBER: 36,749
(C) REFERENCE/DOCKET NUMBER: PF-0484 US

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 650-855-0555
(B) TELEFAX: 650-845-4166

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 619 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: SMCANOT01
(B) CLONE: 2479739

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met	Ser	Gly	Leu	Val	Leu	Gly	Gln	Arg	Asp	Glu	Pro	Ala	Gly	His	Arg
1				5					10					15	
Leu	Ser	Gln	Glu	Glu	Ile	Leu	Gly	Ser	Thr	Arg	Leu	Val	Ser	Gln	Gly
			20					25					30		
Leu	Glu	Ala	Leu	Arg	Ser	Glu	His	Gln	Ala	Val	Leu	Gln	Ser	Leu	Ser
		35					40					45			
Gln	Thr	Ile	Glu	Cys	Leu	Gln	Gln	Gly	Gly	His	Glu	Glu	Gly	Leu	Val
	50					55					60				

His Glu Lys Ala Arg Gln Leu Arg Arg Ser Met Glu Asn Ile Glu Leu
65 70 75 80
Gly Leu Ser Glu Ala Gln Val Met Leu Ala Leu Ala Ser His Leu Ser
85 90 95
Thr Val Glu Ser Glu Lys Gln Lys Leu Arg Ala Gln Val Arg Arg Leu
100 105 110
Cys Gln Glu Asn Gln Trp Leu Arg Asp Glu Leu Ala Gly Thr Gln Gln
115 120 125
Arg Leu Gln Arg Ser Glu Gln Ala Val Ala Gln Leu Glu Glu Glu Lys
130 135 140
Lys His Leu Glu Phe Leu Gly Gln Leu Arg Gln Tyr Asp Glu Asp Gly
145 150 155 160
His Thr Ser Glu Glu Lys Glu Gly Asp Ala Thr Lys Asp Ser Leu Asp
165 170 175
Asp Leu Phe Pro Asn Glu Glu Glu Glu Asp Pro Ser Asn Gly Leu Ser
180 185 190
Arg Gly Gln Gly Ala Thr Ala Ala Gln Gln Gly Gly Tyr Glu Ile Pro
195 200 205
Ala Arg Leu Arg Thr Leu His Asn Leu Val Ile Gln Tyr Ala Ala Gln
210 215 220
Gly Arg Tyr Glu Val Ala Val Pro Leu Cys Lys Gln Ala Leu Glu Asp
225 230 235 240
Leu Glu Arg Thr Ser Gly Arg Gly His Pro Asp Val Ala Thr Met Leu
245 250 255
Asn Ile Leu Ala Leu Val Tyr Arg Asp Gln Asn Lys Tyr Lys Glu Ala
260 265 270
Ala His Leu Leu Asn Asp Ala Leu Ser Ile Arg Glu Ser Thr Leu Gly
275 280 285
Pro Asp His Pro Ala Val Ala Ala Thr Leu Asn Asn Leu Ala Val Leu
290 295 300
Tyr Gly Lys Arg Gly Lys Tyr Lys Glu Ala Glu Pro Leu Cys Gln Arg
305 310 315 320
Ala Leu Glu Ile Arg Glu Lys Val Leu Gly Thr Asn His Pro Asp Val
325 330 335
Ala Lys Gln Leu Asn Asn Leu Ala Leu Leu Cys Gln Asn Gln Gly Lys
340 345 350
Tyr Glu Ala Val Glu Arg Tyr Tyr Gln Arg Ala Leu Ala Ile Tyr Glu
355 360 365
Gly Gln Leu Gly Pro Asp Asn Pro Asn Val Ala Arg Thr Lys Asn Asn
370 375 380
Leu Ala Ser Cys Tyr Leu Lys Gln Gly Lys Tyr Ala Glu Ala Glu Thr
385 390 395 400
Leu Tyr Lys Glu Ile Leu Thr Arg Ala His Val Gln Glu Phe Gly Ser
405 410 415
Val Asp Asp Asp His Lys Pro Ile Trp Met His Ala Glu Glu Arg Glu
420 425 430
Glu Met Ser Lys Ser Arg His His Glu Gly Gly Thr Pro Tyr Ala Glu
435 440 445
Tyr Gly Gly Trp Tyr Lys Ala Cys Lys Val Ser Ser Pro Thr Val Asn
450 455 460
Thr Thr Leu Arg Asn Leu Gly Ala Leu Tyr Arg Arg Gln Gly Lys Leu
465 470 475 480
Glu Ala Ala Glu Thr Leu Glu Glu Cys Ala Leu Arg Ser Arg Arg Gln
485 490 495
Gly Thr Asp Pro Ile Ser Gln Thr Lys Val Ala Glu Leu Leu Gly Glu
500 505 510
Ser Asp Gly Arg Arg Thr Ser Gln Glu Gly Pro Gly Asp Ser Val Lys
515 520 525
Phe Glu Gly Gly Glu Asp Ala Ser Val Ala Val Glu Trp Ser Gly Asp
530 535 540
Gly Ser Gly Thr Leu Gln Arg Ser Gly Ser Leu Gly Lys Ile Arg Asp
545 550 555 560
Val Leu Arg Arg Ser Ser Glu Leu Leu Val Arg Lys Leu Gln Gly Thr
565 570 575
Glu Pro Arg Pro Ser Ser Ser Asn Met Lys Arg Ala Ala Ser Leu Asn
580 585 590
Tyr Leu Asn Gln Pro Ser Ala Ala Pro Leu Gln Val Ser Arg Gly Leu

595 600 605
Ser Ala Ser Thr Met Asp Leu Ser Ser Ser Ser
610 615

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2453 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: SMCANOT01
(B) CLONE: 2479739

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
GTGAAGTGGT GAAAGAAGGG GTGGGAACGC TGGACTTCTG GACTTTGGGC AGGGCAGATC     60
CTCTGACTCT CTGGCTGCAG AACAGTTTCT TCCGTGCTCT GGCCTGAGTG CCCACAGGCC    120
AGGGGCCTCT GCTCTGTACA CAGACCGGGC AAGGTCCCCC AGGCCAGGAT GTCAGGCCTG    180
GTGTTGGGGC AGCGGGATGA GCCTGCAGGC CACCGGCTCA GCCAAGAGGA GATCCTGGGG    240
AGCACACGGC TGGTCAGCCA AGGGCTAGAG GCCCTACGCA GTGAACACCA GGCCGTGCTG    300
CAAAGCCTGT CCCAGACCAT TGAGTGTCTG CAGCAGGGAG GCCATGAGGA AGGGCTGGTG    360
CATGAGAAGG CCCGGCAGCT TCGCCGTTCT ATGGAAAACA TTGAGCTCGG GCTGAGTGAG    420
GCCCAGGTGA TGCTGGCTCT AGCCAGCCAC CTGAGCACAG TGGAGTCGGA GAAACAGAAG    480
CTGCGGGCTC AGGTGCGGCG GCTATGCCAG GAGAACCAGT GGCTGCGGGA TGAGCTGGCT    540
GGCACCCAGC AGCGGCTACA GCGCAGTGAA CAGGCTGTGG CTCAGCTGGA GGAGGAAAAG    600
AAGCACCTGG AGTTCCTGGG GCAGCTGCGG CAGTATGATG AGGATGGACA TACCTCGGAG    660
GAGAAAGAAG GCGATGCCAC CAAGGATTCC CTGGATGACC TCTTTCCTAA TGAGGAGGAA    720
GAGGACCCCA GCAATGGCTT GTCCCGTGGT CAAGGTGCTA CAGCAGCTCA GCAGGGTGGA    780
TATGAGATCC CAGCAAGGTT GCGGACGTTG CACAACCTGG TGATCCAGTA CGCAGCCCAA    840
GGTCGCTATG AGGTGGCCGT GCCACTCTGT AAGCAGGCAC TAGAGGACCT GGAGCGCACA    900
TCAGGCCGTG GCCACCCTGA TGTCGCCACC ATGCTCAACA TCCTTGCTTT GGTGTATCGT    960
GACCAGAATA AGTATAAGGA AGCTGCCCAC CTGCTGAATG ATGCCCTTAG CATCCGGGAG   1020
AGCACCTTGG GACCTGACCA TCCTGCTGTG GCTGCCACAC TCAACAATTT GGCTGTGCTC   1080
TATGGCAAAA GGGGCAAGTA CAAGGAGGCA GAGCCTCTGT GCCAGCGGGC ACTGGAGATT   1140
CGAGAAAAGG TCCTGGGCAC GAATCATCCA GATGTGGCAA AACAGCTGAA CAACCTGGCC   1200
CTCTTGTGCC AAAACCAGGG CAAGTATGAG GCCGTGGAAC GCTACTACCA GCGAGCACTG   1260
GCCATCTACG AGGGGCAGCT GGGGCCGGAC AACCCTAATG TAGCCCGGAC CAAGAACAAC   1320
CTGGCTTCCT GTTACCTGAA ACAGGGCAAA TATGCTGAGG CTGAGACACT ATACAAAGAG   1380
ATCCTGACCC GTGCCCATGT ACAGGAGTTT GGGTCTGTGG ATGATGACCA CAAGCCCATC   1440
TGGATGCATG CAGAGGAGCG GGAGGAAATG AGCAAAAGCC GGCACCATGA GGGTGGGACA   1500
CCCTATGCTG AGTATGGAGG CTGGTACAAG GCCTGCAAAG TGAGCAGCCC CACAGTGAAC   1560
ACTACTCTGA GAAACCTGGG AGCTCTGTAT AGGCGCCAGG GAAAGCTGGA GGCTGCTGAG   1620
ACCCTGGAGG AATGTGCCCT GCGGTCCCGG AGACAGGGCA CTGACCCTAT CAGCCAGACG   1680
AAGGTGGCAG AGCTGCTTGG GGAGAGTGAT GGTAGAAGGA CCTCCCAGGA GGGCCCTGGA   1740
GACAGTGTGA AATTCGAGGG TGGTGAAGAT GCTTCTGTGG CTGTGGAGTG GTCCGGGGAT   1800
GGCAGTGGGA CCCTGCAGAG GAGTGGCTCT CTTGGCAAGA TCCGGGATGT GCTCCGCAGA   1860
AGCAGTGAAC TCTTGGTGAG GAAGCTCCAG GGGACTGAGC CTCGGCCCTC CAGCAGCAAC   1920
ATGAAGCGAG CAGCCTCCTT GAACTATCTG AACCAACCTA GTGCAGCACC CCTCCAGGTC   1980
TCCCGGGGCC TCAGTGCCAG CACCATGGAC CTCTCTTCAA GCAGCTGACA TTCAACCCGG   2040
CCCCCAGGTC TGCTGGGTCC CCCCACCCCC ACAGCCCTCA CAGCATTCCC CATTGCTCCT   2100
GGCTCTTCCC CACCCCTAGG TGGGACAGTG AAGGGGAGCA GTTTAACCAG AAGATTGCTG   2160
CTGCCCTTAG GGTCTCAGCT CCCTCCTCAG GAATCCCTCT TAGGAAGGAC CCTCAGGACA   2220
CCCTCTCTGC ACCCTGTGGT CCTCTAGAGT AGCTAGCTCT GAGGCCCCAA GGTGGGTACA   2280
AAGCAGGTAT GGCCCTCAGA GATGCAGCCT GCTGCTGGCT TTTCAGTCAG AGGGTTGGGG   2340
GCTGGCCAGC CAAGCTGCCT TGCCCTGGCC GCTCTTACTC CCTCCCTCTG CTGTCTCACT   2400
TCAGGTCCAT GTATTTCACT TTTCTTAAAT AAAAGAATCA GTNCTTNTNT NNG          2453
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 569 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: GenBank
(B) CLONE: 307085

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Ser Thr Met Val Tyr Ile Lys Glu Asp Lys Leu Glu Lys Leu Thr
 1               5                  10                  15
Gln Asp Glu Ile Ile Ser Lys Thr Lys Gln Val Ile Gln Gly Leu Glu
            20                  25                  30
Ala Leu Lys Asn Glu His Asn Ser Ile Leu Gln Ser Leu Leu Glu Thr
        35                  40                  45
Leu Lys Cys Leu Lys Lys Asp Asp Glu Ser Asn Leu Val Glu Glu Lys
    50                  55                  60
Ser Asn Met Ile Arg Lys Ser Leu Glu Met Leu Glu Leu Gly Leu Ser
65                  70                  75                  80
Glu Ala Gln Val Met Met Ala Leu Ser Asn His Leu Asn Ala Val Glu
                85                  90                  95
Ser Glu Lys Gln Lys Leu Arg Ala Gln Val Arg Arg Leu Cys Gln Glu
            100                 105                 110
Asn Gln Trp Leu Arg Asp Glu Leu Ala Asn Thr Gln Gln Lys Leu Gln
        115                 120                 125
Lys Ser Glu Gln Ser Val Ala Gln Leu Glu Glu Glu Lys Lys His Leu
    130                 135                 140
Glu Phe Met Asn Gln Leu Lys Lys Tyr Asp Asp Asp Ile Ser Pro Ser
145                 150                 155                 160
Glu Asp Lys Asp Thr Asp Ser Thr Lys Glu Pro Leu Asp Asp Leu Phe
                165                 170                 175
Pro Asn Asp Glu Asp Asp Pro Gly Gln Gly Ile Gln Gln Gln His Ser
            180                 185                 190
Ser Ala Ala Ala Ala Ala Gln Gln Gly Gly Tyr Glu Ile Pro Ala Arg
        195                 200                 205
Leu Arg Thr Leu His Asn Leu Val Ile Gln Tyr Ala Ser Gln Gly Arg
    210                 215                 220
Tyr Glu Val Ala Val Pro Leu Cys Lys Gln Ala Leu Glu Asp Leu Glu
225                 230                 235                 240
Lys Thr Ser Gly His Asp His Pro Asp Val Ala Thr Met Leu Asn Ile
                245                 250                 255
Leu Ala Leu Val Tyr Arg Asp Gln Asn Lys Tyr Lys Asp Ala Ala Asn
            260                 265                 270
Leu Leu Asn Asp Ala Leu Ala Ile Arg Glu Lys Thr Leu Gly Lys Asp
        275                 280                 285
His Pro Ala Val Ala Ala Thr Leu Asn Asn Leu Ala Val Leu Tyr Gly
    290                 295                 300
Lys Arg Gly Lys Tyr Lys Glu Ala Glu Pro Leu Cys Lys Arg Ala Leu
305                 310                 315                 320
Glu Ile Arg Glu Lys Val Leu Gly Lys Asp His Pro Asp Val Ala Lys
                325                 330                 335
Gln Leu Asn Asn Leu Ala Leu Leu Cys Gln Asn Gln Gly Lys Tyr Glu
            340                 345                 350
Glu Val Glu Tyr Tyr Tyr Gln Arg Ala Leu Glu Ile Tyr Gln Thr Lys
        355                 360                 365
Leu Gly Pro Asp Asp Pro Asn Val Ala Lys Thr Lys Asn Asn Leu Ala
    370                 375                 380
Ser Cys Tyr Leu Lys Gln Gly Lys Phe Lys Gln Ala Glu Thr Leu Tyr
385                 390                 395                 400
Lys Glu Ile Leu Thr Arg Ala His Glu Arg Glu Phe Gly Ser Val Asp
                405                 410                 415
Asp Glu Asn Lys Pro Ile Trp Met His Ala Glu Glu Arg Glu Glu Cys
            420                 425                 430
Lys Gly Lys Gln Lys Asp Gly Thr Ser Phe Gly Glu Tyr Gly Gly Trp
        435                 440                 445
Tyr Lys Ala Cys Lys Val Asp Ser Pro Thr Val Thr Thr Thr Leu Lys
    450                 455                 460
Asn Leu Gly Ala Leu Tyr Arg Arg Gln Gly Lys Phe Glu Ala Ala Glu
465                 470                 475                 480
Thr Leu Glu Glu Ala Ala Met Arg Ser Arg Lys Gln Gly Leu Asp Asn
```

```
                485                       490                       495
Val His Lys Gln Arg Val Ala Glu Val Leu Asn Asp Pro Glu Asn Met
            500                     505                     510
Glu Lys Arg Arg Ser Arg Glu Ser Leu Asn Val Asp Val Val Lys Tyr
        515                     520                     525
Glu Ser Gly Pro Asp Gly Gly Glu Glu Val Ser Met Ser Val Glu Trp
    530                     535                     540
Asn Gly Gly Val Ser Gly Arg Ala Ser Phe Cys Gly Lys Arg Gln Gln
545                     550                     555                     560
Gln Gln Trp Pro Gly Arg Arg His Arg
                565
```

What is claimed is:

1. A substantially purified polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

2. A substantially purified variant having at least 90% amino acid sequence identity to the sequence of claim 1.

3. An isolated and purified polynucleotide encoding the polypeptide of claim 1.

4. An isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide of claim 3.

5. An isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide of claim 3.

6. An isolated and purified polynucleotide which is complementary to the polynucleotide of claim 3.

7. An isolated and purified polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2 or a fragment of SEQ ID NO:2.

8. An isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide of claim 7.

9. An isolated and purified polynucleotide having a sequence complementary to the polynucleotide of claim 7.

10. An expression vector containing at least a fragment of the polynucleotide of claim 3.

11. A host cell containing the expression vector of claim 10.

12. A method for producing a polypeptide comprising a sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, the method comprising the steps of:

AB3

(a) culturing the host cell of claim 11 under conditions suitable for the expression of the polypeptide; and

(b) recovering the polypeptide from the host cell culture.

13. A pharmaceutical composition comprising the polypeptide of claim 1 in conjunction with a suitable pharmaceutical carrier.

14. A purified antibody which specifically binds to the polypeptide of claim 1.

15. A purified agonist of the polypeptide of claim 1.

16. A purified antagonist of the polypeptide of claim 1.

17. A method for treating or preventing a neurological disorder, the method comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 13.

18. A method for treating or preventing a reproductive disorder, the method comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 13.

19. A method for treating or preventing a cell proliferative disorder, the method comprising administering to a subject in need of such treatment an effective amount of the pharmaceutical composition of claim 13.

20. A method for detecting a polynucleotide encoding a polypeptide comprising

the amino acid sequence of SEQ ID NO:1 in a biological sample containing nucleic acids, the method comprising the steps of:

(a) hybridizing the polynucleotide of claim 6 to at least one of the nucleic acids of the biological sample, thereby forming a hybridization complex; and

(b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding the polypeptide in the biological sample.

21. The method of claim 20 wherein the nucleic acids of the biological sample are amplified by the polymerase chain reaction prior to the hybridizing step.

add

ABSTRACT OF THE DISCLOSURE

The invention provides a human kinesin light chain homolog (KILCH) and polynucleotides which identify and encode KILCH. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for treating or preventing disorders associated with expression of KILCH.

```
                9           18          27          36          45          54
5' GTG AAG TGG TGA AAG AAG GGG TGG GAA CGC TGG ACT TCT GGA CTT TGG GCA GGG

               63          72          81          90          99         108
   CAG ATC CTC TGA CTC TCT GGC TGC AGA ACA GTT TCT TCC GTG CTC TGG CCT GAG

              117         126         135         144         153         162
   TGC CCA CAG GCC AGG GGC CTC TGC TCT GTA CAC AGA CCG GGC AAG GTC CCC CAG

              171         180         189         198         207         216
   GCC AGG ATG TCA GGC CTG GTG TTG GGG CAG CGG GAT GAG CCT GCA GGC CAC CGG
           M   S   G   L   V   L   G   Q   R   D   E   P   A   G   H   R

              225         234         243         252         261         270
   CTC AGC CAA GAG GAG ATC CTG GGG AGC ACA CGG CTG GTC AGC CAA GGG CTA GAG
   L   S   Q   E   E   I   L   G   S   T   R   L   V   S   Q   G   L   E

              279         288         297         306         315         324
   GCC CTA CGC AGT GAA CAC CAG GCC GTG CTG CAA AGC CTG TCC CAG ACC ATT GAG
   A   L   R   S   E   H   Q   A   V   L   Q   S   L   S   Q   T   I   E

              333         342         351         360         369         378
   TGT CTG CAG CAG GGA GGC CAT GAG GAA GGG CTG GTG CAT GAG AAG GCC CGG CAG
   C   L   Q   Q   G   G   H   E   E   G   L   V   H   E   K   A   R   Q

              387         396         405         414         423         432
   CTT CGC CGT TCT ATG GAA AAC ATT GAG CTC GGG CTG AGT GAG GCC CAG GTG ATG
   L   R   R   S   M   E   N   I   E   L   G   L   S   E   A   Q   V   M
```

FIGURE 1A

```
        441         450         459         468         477         486
CTG GCT CTA GCC AGC CAC CTG AGC ACA GTG GAG TCG GAG AAA CAG AAG CTG CGG
L   A   L   A   S   H   L   S   T   V   E   S   E   K   Q   K   L   R

        495         504         513         522         531         540
GCT CAG GTG CGG CGG CTA TGC CAG GAG AAC CAG TGG CTG CGG GAT GAG CTG GCT
A   Q   V   R   R   L   C   Q   E   N   Q   W   L   R   D   E   L   A

        549         558         567         576         585         594
GGC ACC CAG CAG CGG CTA CAG CGC AGT GAA CAG GCT GTG GCT CAG CTG GAG GAG
G   T   Q   Q   R   L   Q   R   S   E   Q   A   V   A   Q   L   E   E

        603         612         621         630         639         648
GAA AAG AAG CAC CTG GAG TTC CTG GGG CAG CTG CGG CAG TAT GAT GAG GAT GGA
E   K   K   H   L   E   F   L   G   Q   L   R   Q   Y   D   E   D   G

        657         666         675         684         693         702
CAT ACC TCG GAG GAG AAA GAA GGC GAT GCC ACC AAG GAT TCC CTG GAT GAC CTC
H   T   S   E   E   K   E   G   D   A   T   K   D   S   L   D   D   L

        711         720         729         738         747         756
TTT CCT AAT GAG GAG GAA GAG GAC CCC AGC AAT GGC TTG TCC CGT GGT CAA GGT
F   P   N   E   E   E   E   D   P   S   N   G   L   S   R   G   Q   G

        765         774         783         792         801         810
GCT ACA GCA GCT CAG CAG GGT GGA TAT GAG ATC CCA GCA AGG TTG CGG ACG TTG
A   T   A   A   Q   Q   G   G   Y   E   I   P   A   R   L   R   T   L
```

FIGURE 1B

```
                819                 828                 837                 846                 855                 864
CAC AAC CTG GTG ATC CAG TAC GCA GCC CAA GGT CGC TAT GAG GTG GCC GTG CCA
H   N   L   V   I   Q   Y   A   A   Q   G   R   Y   E   V   A   V   P

                873                 882                 891                 900                 909                 918
CTC TGT AAG CAG GCA CTA GAG GAC CTG GAG CGC ACA TCA GGC CGT GGC CAC CCT
L   C   K   Q   A   L   E   D   L   E   R   T   S   G   R   G   H   P

                927                 936                 945                 954                 963                 972
GAT GTC GCC ACC ATG CTC AAC ATC CTT GCT TTG GTG TAT CGT GAC CAG AAT AAG
D   V   A   T   M   L   N   I   L   A   L   V   Y   R   D   Q   N   K

                981                 990                 999                1008                1017                1026
TAT AAG GAA GCT GCC CAC CTG CTG AAT GAT GCC CTT AGC ATC CGG GAG AGC ACC
Y   K   E   A   A   H   L   L   N   D   A   L   S   I   R   E   S   T

               1035                1044                1053                1062                1071                1080
TTG GGA CCT GAC CAT CCT GCT GTG GCT GCC ACA CTC AAC AAT TTG GCT GTG CTC
L   G   P   D   H   P   A   V   A   A   T   L   N   N   L   A   V   L

               1089                1098                1107                1116                1125                1134
TAT GGC AAA AGG GGC AAG TAC AAG GAG GCA GAG CCT CTG TGC CAG CGG GCA CTG
Y   G   K   R   G   K   Y   K   E   A   E   P   L   C   Q   R   A   L

               1143                1152                1161                1170                1179                1188
GAG ATT CGA GAA AAG GTC CTG GGC ACG AAT CAT CCA GAT GTG GCA AAA CAG CTG
E   I   R   E   K   V   L   G   T   N   H   P   D   V   A   K   Q   L
```

FIGURE 1C

APPROVED BY DRAFTSMAN	O.G. FIG.	
	CLASS	SUBCLASS

```
        1197         1206        1215         1224         1233         1242
AAC AAC CTG GCC CTC TTG TGC CAA AAC CAG GGC AAG TAT GAG GCC GTG GAA CGC
N   N   L   A   L   L   C   Q   N   Q   G   K   Y   E   A   V   E   R

        1251         1260        1269         1278         1287         1296
TAC TAC CAG CGA GCA CTG GCC ATC TAC GAG GGG CAG CTG GGG CCG GAC AAC CCT
Y   Y   Q   R   A   L   A   I   Y   E   G   Q   L   G   P   D   N   P

        1305         1314        1323         1332         1341         1350
AAT GTA GCC CGG ACC AAG AAC AAC CTG GCT TCC TGT TAC CTG AAA CAG GGC AAA
N   V   A   R   T   K   N   N   L   A   S   C   Y   L   K   Q   G   K

        1359         1368        1377         1386         1395         1404
TAT GCT GAG GCT GAG ACA CTA TAC AAA GAG ATC CTG ACC CGT GCC CAT GTA CAG
Y   A   E   A   E   T   L   Y   K   E   I   L   T   R   A   H   V   Q

        1413         1422        1431         1440         1449         1458
GAG TTT GGG TCT GTG GAT GAT GAC CAC AAG CCC ATC TGG ATG CAT GCA GAG GAG
E   F   G   S   V   D   D   D   H   K   P   I   W   M   H   A   E   E

        1467         1476        1485         1494         1503         1512
CGG GAG GAA ATG AGC AAA AGC CGG CAC CAT GAG GGT GGG ACA CCC TAT GCT GAG
R   E   E   M   S   K   S   R   H   H   E   G   G   T   P   Y   A   E

        1521         1530        1539         1548         1557         1566
TAT GGA GGC TGG TAC AAG GCC TGC AAA GTG AGC AGC CCC ACA GTG AAC ACT ACT
Y   G   G   W   Y   K   A   C   K   V   S   S   P   T   V   N   T   T
```

FIGURE 1D

```
         1575                1584                1593                1602                1611                1620
CTG AGA AAC CTG GGA GCT CTG TAT AGG CGC CAG GGA AAG CTG GAG GCT GCT GAG
L   R   N   L   G   A   L   Y   R   R   Q   G   K   L   E   A   A   E

         1629                1638                1647                1656                1665                1674
ACC CTG GAG GAA TGT GCC CTG CGG TCC CGG AGA CAG GGC ACT GAC CCT ATC AGC
T   L   E   E   C   A   L   R   S   R   R   Q   G   T   D   P   I   S

         1683                1692                1701                1710                1719                1728
CAG ACG AAG GTG GCA GAG CTG CTT GGG GAG AGT GAT GGT AGA AGG ACC TCC CAG
Q   T   K   V   A   E   L   L   G   E   S   D   G   R   R   T   S   Q

         1737                1746                1755                1764                1773                1782
GAG GGC CCT GGA GAC AGT GTG AAA TTC GAG GGT GGT GAA GAT GCT TCT GTG GCT
E   G   P   G   D   S   V   K   F   E   G   G   E   D   A   S   V   A

         1791                1800                1809                1818                1827                1836
GTG GAG TGG TCC GGG GAT GGC AGT GGG ACC CTG CAG AGG AGT GGC TCT CTT GGC
V   E   W   S   G   D   G   S   G   T   L   Q   R   S   G   S   L   G

         1845                1854                1863                1872                1881                1890
AAG ATC CGG GAT GTG CTC CGC AGA AGC AGT GAA CTC TTG GTG AGG AAG CTC CAG
K   I   R   D   V   L   R   R   S   S   E   L   L   V   R   K   L   Q

         1899                1908                1917                1926                1935                1944
GGG ACT GAG CCT CGG CCC TCC AGC AGC AAC ATG AAG CGA GCA GCC TCC TTG AAC
G   T   E   P   R   P   S   S   S   N   M   K   R   A   A   S   L   N
```

FIGURE 1E

```
          1953           1962           1971           1980           1989           1998
TAT CTG AAC CAA CCT AGT GCA GCA CCC CTC CAG GTC TCC CGG GGC CTC AGT GCC
Y   L   N   Q   P   S   A   A   P   L   Q   V   S   R   G   L   S   A

          2007           2016           2025           2034           2043           2052
AGC ACC ATG GAC CTC TCT TCA AGC AGC TGA CAT TCA ACC CGG CCC CCA GGT CTG
S   T   M   D   L   S   S   S   S

          2061           2070           2079           2088           2097           2106
CTG GGT CCC CCC ACC CCC ACA GCC CTC ACA GCA TTC CCC ATT GCT CCT GGC TCT

          2115           2124           2133           2142           2151           2160
TCC CCA CCC CTA GGT GGG ACA GTG AAG GGG AGC AGT TTA ACC AGA AGA TTG CTG

          2169           2178           2187           2196           2205           2214
CTG CCC TTA GGG TCT CAG CTC CCT CCT CAG GAA TCC CTC TTA GGA AGG ACC CTC

          2223           2232           2241           2250           2259           2268
AGG ACA CCC TCT CTG CAC CCT GTG GTC CTC TAG AGT AGC TAG CTC TGA GGC CCC

          2277           2286           2295           2304           2313           2322
AAG GTG GGT ACA AAG CAG GTA TGG CCC TCA GAG ATG CAG CCT GCT GCT GGC TTT

          2331           2340           2349           2358           2367           2376
TCA GTC AGA GGG TTG GGG GCT GGC CAG CCA AGC TGC CTT GCC CTG GCC GCT CTT

          2385           2394           2403           2412           2421           2430
ACT CCC TCC CTC TGC TGT CTC ACT TCA GGT CCA TGT ATT TCA CTT TTC TTA AAT
```

FIGURE 1F

```
        2439          2448
AAA AGA ATC AGT NCT TNT NTN NG 3'
```

FIGURE 1G

```
1    MSGLVLGQRDEPAGHRLSQEEILGSTRLVS  2479739
1    MSTMVYIKEDKL--EKLTQDEIISKTKQVI  GI 307085

31   QGLEALRSEHQAVLQSLSQTIECLQQGGHE  2479739
29   QGLEALKNEHNSILQSLLETLKCLKKDD-E  GI 307085

61   EGLVHEKARQLRRSMENIELGLSEAQVMLA  2479739
58   SNLVEEKSNMIRKSLEMLELGLSEAQVMMA  GI 307085

91   LASHLSTVESEKQKLRAQVRRLCQENQWLR  2479739
88   LSNHLNAVESEKQKLRAQVRRLCQENQWLR  GI 307085

121  DELAGTQQRLQRSEQAVAQLEEEKKHLEFL  2479739
118  DELANTQQKLQKSEQSVAQLEEEKKHLEFM  GI 307085

151  GQLRQYDEDGHTSEEKEGDATKDSLDDLFP  2479739
148  NQLKKYDDDISPSEDKDTDSTKEPLDDLFP  GI 307085

181  NEEEEDPSNGLSRGQG--ATAAQQGGYEIP  2479739
178  NDED-DPGQGIQQQHSSAAAAAQQGGYEIP  GI 307085
```

FIGURE 2A

```
209 ARLRTLHNLVIQYAAQGRYEVAVPLCKQAL 2479739
207 ARLRTLHNLVIQYASQGRYEVAVPLCKQAL GI 307085

239 EDLERTSGRGHPDVATMLNILALVYRDQNK 2479739
237 EDLEKTSGHDHPDVATMLNILALVYRDQNK GI 307085

269 YKEAAHLLNDALSIRESTLGPDHPAVAATL 2479739
267 YKDAANLLNDALAIREKTLGKDHPAVAATL GI 307085

299 NNLAVLYGKRGKYKEAEPLCQRALEIREKV 2479739
297 NNLAVLYGKRGKYKEAEPLCKRALEIREKV GI 307085

329 LGTNHPDVAKQLNNLALLCQNQGKYEAVER 2479739
327 LGKDHPDVAKQLNNLALLCQNQGKYEEVEY GI 307085

359 YYQRALAIYEGQLGPDNPNVARTKNNLASC 2479739
357 YYQRALEIYQTKLGPDDPNVAKTKNNLASC GI 307085

389 YLKQGKYAEAETLYKEILTRAHVQEFGSVD 2479739
387 YLKQGKFKQAETLYKEILTRAHEREFGSVD GI 307085
```

FIGURE 2B

```
419  D D H K P I W M H A E E R E E M S K S R H H E G G T P Y A E   2479739
417  D E N K P I W M H A E E R E E - C K G K Q K D G - T S F G E   GI 307085

449  Y G G W Y K A C K V S S P T V N T T L R N L G A L Y R R Q G   2479739
445  Y G G W Y K A C K V D S P T V T T T L K N L G A L Y R R Q G   GI 307085

479  K L E A A E T L E E C A L R S R R Q G T D P I S Q T K V A E   2479739
475  K F E A A E T L E E A A M R S R K Q G L D N V H K Q R V A E   GI 307085

509  L L G E S D G - - - R R T S Q E G P G D S V K F E - - - - G   2479739
505  V L N D P E N M E K R R S R E S L N V D V V K Y E S G P D G   GI 307085

532  G E D A S V A V E W S G D G S G T L Q R S G S L G K I R D V   2479739
535  G E E V S M S V E W N G G V S G - - - R A S F C G K R Q Q -   GI 307085

562  L R R S S E L L V R K L Q G T E P R P S S S N M K R A A S L   2479739
561  - - - - - - - - - - - - - - - Q Q W P G R R H R               GI 307085

592  N Y L N Q P S A A P L Q V S R G L S A S T M D L S S S S       2479739
569                                                                GI 307085
```

FIGURE 2C

Docket No.: PF-0484 US

DECLARATION AND POWER OF ATTORNEY FOR UNITED STATES PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a United States patent is sought on the invention entitled

KINESIN LIGHT CHAIN HOMOLOG

the specification of which:

/ X / is attached hereto.

/___/ was filed on ______________ as application Serial No. ______________ and if this box contains an X /___/, was amended on ______________________________.

/___/ was filed as Patent Cooperation Treaty international application No. __________ on _______, 19__, if this box contains an X /_/, was amended on under Patent Cooperation Treaty Article 19 on _______19_, and if this box contains an X /_/, was amended on _______________.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge my duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim the benefit under Title 35, United States Code, §119 or §365(a)-(b) of any foreign application(s) for patent or inventor's certificate indicated below and of any Patent Cooperation Treaty international applications(s) designating at least one country other than the United States indicated below and have also identified below any foreign application(s) for patent or inventor's certificate and Patent Cooperation Treaty international application(s) designating at least one country other than the United States for the same subject matter and having a filing date before that of the application for said subject matter the priority of which is claimed:

Docket No.: PF-0484 US

Country	Number	Filing Date	Priority Claimed
			/_/ Yes /_/ No
			/_/ Yes /_/ No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in said prior application(s) in the manner required by the first paragraph of Title 35, United States Code §112, I acknowledge my duty to disclose material information as defined in Title 37 Code of Federal Regulations, §1.56(a) which occurred between the filing date(s) of the prior application(s) and the national or Patent Cooperation Treaty international filing date of this application:

Application Serial No.	Filed	Status (Pending, Abandoned, Patented)

I hereby appoint the following:

LUCY J. BILLINGS	**Registration No. 36,749**
MICHAEL C. CERRONE	**Registration No. 39,132**
SHEELA MOHAN-PETERSON	**Registration No. 41,201**
COLETTE C. MUENZEN	**Registration No. 39,784**
KAREN J. ZELLER	**Registration No. 37,071**

respectively and individually, as my attorneys and/or agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. Please address all communications to:

LUCY J. BILLINGS, ESQ.
INCYTE PHARMACEUTICALS, INC.
3174 PORTER DRIVE, PALO ALTO, CA 94304

TEL: 650-855-0555 FAX: 650-845-4166

I hereby declare that all statements made herein of my own knowledge are true and that all

statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Sole Inventor or First Joint Inventor:	Full name:	**JENNIFER L. HILLMAN**
	Signature:	
	Date:	February 27, 1998
	Citizenship:	United States of America
	Residence:	Mountain View, California
	P.O. Address:	230 Monroe Drive, #12 Mountain View, California
Second Joint Inventor:	Full name:	**PREETI LAL**
	Signature:	
	Date:	Feb 27th, 1998
	Citizenship:	India
	Residence:	Santa Clara, California
	P.O. Address:	2382 Lass Drive Santa Clara, California, 95054

Third Joint Inventor:	Full name:	**Y. TOM TANG**
	Signature:	
	Date:	March 3, 1998
	Citizenship:	People's Republic of China
	Residence:	San Jose, California
	P.O. Address:	4230 Ranwick Court San Jose, California, 95118
Fourth Joint Inventor:	Full name:	**HENRY YUE**
	Signature:	
	Date:	2-27, 1998
	Citizenship:	United States of America
	Residence:	Sunnyvale, California
	P.O. Address:	826 Lois Avenue Sunnyvale, California, 94087
Fifth Joint Inventor:	Full name:	**NEIL C. CORLEY**
	Signature:	
	Date:	3-2, 1998
	Citizenship:	United States of America
	Residence:	Mountain View, California
	P.O. Address:	1240 Dale Avenue, #30 Mountain View, California, 94040